of nanoparticles (see Figure 28B) so that the two types of nanoparticles hybridize to each other to form the aggregate probe. Since each type of nanoparticles has a plurality of oligonucleotides attached to it, each type of nanoparticles will hybridize to a plurality of the other type of nanoparticles to form an aggregate containing numerous nanoparticles of both types.

The aggregate probe can be utilized to detect nucleic acid in any of the above assay formats performed on a substrate, eliminating the need to build up layers of individual nanoparticles in order to obtain or enhance a detectable change. To even further enhance the detectable change, layers of aggregate probes can be built up by using two types of aggregate probes, the first type of aggregate probe having oligonucleotides attached to it that are complementary to oligonucleotides on the other type of aggregate probe. In particular, when the aggregate probe is prepared as illustrated in Figure 28B, the aggregate probes can hybridize to each other to form the multiple layers. Some of the possible assay formats utilizing aggregate probes are illustrated in Figures 28C-D. For instance, a type of oligonucleotides comprising sequence c is attached to a substrate (see Figure 28C). Sequence c is complementary to the sequence c' of a portion of a nucleic acid to be detected. The target nucleic acid is added and allowed to hybridize to the oligonucleotides attached to the substrate, after which the aggregate probe is added and allowed to hybridize to the portion of the target nucleic acid having sequence b', thereby producing a detectable change. Alternatively, the target nucleic acid can first be hybridized to the aggregate probe in solution and subsequently hybridized to the oligonucleotides on the substrate, or the target nucleic acid can simultaneously be hybridized to the aggregate probe and the oligonucleotides on the substrate. In another embodiment, the target nucleic acid is allowed to react with the aggregate probe and another type of nanoparticles in solution (see Figure 28D). Some of the oligonucleotides attached to this additional type of nanoparticles comprise sequence c so that they hybridize to sequence c' of the target nucleic acid and some of the oligonucleotides attached to this additional type of nanoparticles comprise sequence d so that they can

subsequently hybridize to oligonucleotides comprising sequence d' which are attached to the substrate.

The core itself can also be used as a probe to detect nucleic acids. One possible assay format is illustrated in Figure 28E. As illustrated there, a type of oligonucleotides comprising sequence b is attached to a substrate. Sequence b is complementary to the sequence b' of a portion of a nucleic acid to be detected. The target nucleic acid is contacted with the substrate and allowed to hybridize to the oligonucleotides attached to the substrate. Then, another type of nanoparticles is added. Some of the oligonucleotides attached to this additional type of nanoparticles comprise sequence c so which is complementary to sequence c' of the target nucleic acid so that the nanoparticles hybridize to the target nucleic acid bound to the substrate. Some of the oligonucleotides attached to the additional type of nanoparticles comprise sequence a or a' complementary to sequences a and a' on the core probe, and the core probe is added and allowed to hybridize to the oligonucleotides on the nanoparticles. Since each core probe has sequences a and a' attached to the nanoparticles which comprise the core, the core probes can hybridize to each other to form multiple layers attached to the substrate, providing a greatly enhanced detectable change. In alternative embodiments, the target nucleic acid could be contacted with the additional type of nanoparticles in solution prior to being contacted with the substrate, or the target nucleic acid, the nanoparticles and the substrate could all be contacted simultaneously. In yet another alternative embodiment, the additional type of nanoparticles could be replaced by a linking oligonucleotide comprising both sequences c and a or a'.

When a substrate is employed, a plurality of the initial types of nanoparticle-oligonucleotide conjugates or oligonucleotides can be attached to the substrate in an array for detecting multiple portions of a target nucleic acid, for detecting multiple different nucleic acids, or both. For instance, a substrate may be provided with rows of spots, each spot containing a different type of oligonucleotide or oligonucleotide-nanoparticle conjugate designed to bind to a portion of a target nucleic acid. A sample containing one or more nucleic acids is applied to each spot, and the rest of the assay is performed in one of the ways

described above using appropriate oligonucleotide-nanoparticle conjugates, oligonucleotide-liposome conjugates, aggregate probes, core probes, and binding oligonucleotides.

Finally, when a substrate is employed, a detectable change can be produced or further enhanced by silver staining. Silver staining can be employed with any type of nanoparticles that catalyze the reduction of silver. Preferred are nanoparticles made of noble metals (*e.g.*, gold and silver). See Bassell, et al., *J. Cell Biol.*, **126**, 863-876 (1994); Braun-Howland et al., *Biotechniques*, **13**, 928-931 (1992). If the nanoparticles being employed for the detection of a nucleic acid do not catalyze the reduction of silver, then silver ions can be complexed to the nucleic acid to catalyze the reduction. See Braun et al., *Nature*, **391**, 775 (1998). Also, silver stains are known which can react with the phosphate groups on nucleic acids.

Silver staining can be used to produce or enhance a detectable change in any assay performed on a substrate, including those described above. In particular, silver staining has been found to provide a huge increase in sensitivity for assays employing a single type of nanoparticle, such as the one illustrated in Figure 25A, so that the use of layers of nanoparticles, aggregate probes and core probes can often be eliminated.

In assays for detecting nucleic acids performed on a substrate, the detectable change can be observed with an optical scanner. Suitable scanners include those used to scan documents into a computer which are capable of operating in the reflective mode (*e.g.*, a flatbed scanner), other devices capable of performing this function or which utilize the same type of optics, any type of greyscale-sensitive measurement device, and standard scanners which have been modified to scan substrates according to the invention (*e.g.*, a flatbed scanner modified to include a holder for the substrate) (to date, it has not been found possible to use scanners operating in the transmissive mode). The resolution of the scanner must be sufficient so that the reaction area on the substrate is larger than a single pixel of the scanner. The scanner can be used with any substrate, provided that the detectable change produced by the assay can be observed against the substrate (*e.g.*, a grey spot, such as that produced by silver staining, can be observed against a white background, but cannot be observed against a grey background). The scanner can be a black-and-white scanner or, preferably,